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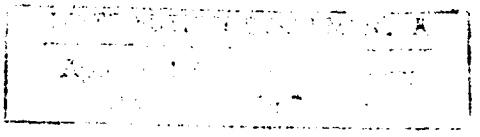

Identification of Specific Protein Markers that Correlate with Initiation and Progression of Microbially Influenced Corrosion

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Abstract

Attempts to resolve proteins in extracts of pipeline pigging solids into its individual protein components by high resolution two dimensional polyacrylamide gel electrophoresis (2-D PAGE) have so far, been unsuccessful. Problems have been encountered during isoelectric focusing in the first dimension. Under the conditions employed, no resolution of the proteins into individual components was obtained. This could be attributed to presence of interfering ions in the samples, inappropriate ampholyte composition or inadequate sample solubilization conditions. Appropriate steps will be taken to correct these problems and optimize conditions for 2-D PAGE. This will include modifications in sample preparation procedures to remove potential interfering ions in isoelectric focusing as well as modifications in gel and sample buffer formulations.

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A. Introduction

The objective of this research is to identify specific protein markers that correlate with initiation and progression of microbially influenced corrosion (MIC). Identification of such markers will facilitate the development of a rapid and economical diagnostic procedure for early detection of MIC. The previous progress report (May 31, 1992) described the development of a screening method for detection of proteins in pipeline pigging solid samples by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), a technique that separates proteins according to their molecular weight (objective 1 of proposal). Procedures for sample preparation, SDS-PAGE, and protein detection as well as results obtained, were described. This report covers work done to accomplish objective 2, which is the detection of individual proteins in the samples by high resolution two dimensional polyacrylamide gel electrophoresis (2-D PAGE).

B. Methods

Protein extracts from the pigging solid samples used for SDS-PAGE were used for 2-D PAGE. The procedure recommended by Bio-Rad for use of the Mini-Protean 2-D cell which was essentially based on O'Farrell's technique was followed. The composition of the first dimension gel solution, sample buffer, overlay buffer and electrode buffers are shown in Table 1. The protein extracts were diluted with first dimension sample buffer (2-4 fold) such that approximately 5-10 μg of protein could be loaded on the tube gel in 5-25 μl volumes. Electrofocusing in the first dimension was carried out at 750 volts for four hours. SDS-PAGE in the second dimension was carried out by loading the tube gel onto the slab gel following a ten minute equilibration in SDS sample reducing buffer. Electrophoresis in the second dimension and staining with silver was carried out as previously described for SDS-PAGE (May, 1992 progress report).

C. Results and Discussion

The samples that have shown detectable proteins on SDS-PAGE were ran on 2-D gels. The results showed problems in electrofocusing in the first dimension. Using the ampholyte composition shown in Table 1, no focusing of the proteins in the extracts from the pipeline pigging solids was achieved. Protein bands were detected in the second dimension gel slab instead of individual protein spots. On the other hand, an *E. coli* extract showed reasonably good focusing and resolution of the proteins into individual spots, under the same conditions. It is possible that the extracts from the pigging solids have too much SDS and other ions that interfere with the focusing.

The pigging solids do contain a high proportion of metal sulfides, black particles which can be dissolved by treatment with acid. Alternatively, the pH gradient (ampholyte composition) used may not be optimum for these samples. Another reason could be precipitation of the proteins at their isoelectric pH or just poor solubility at lower detergent concentrations.

It is apparent from the results that optimum conditions for 2-D gel electrophoresis need to be worked out. It may be necessary to modify the sample preparation procedures to make the protein extracts more suitable for isoelectric focusing in the first dimension. We have begun incorporating a dialysis and desalting step into the sample preparation. Desalting will be accomplished by passage of the sample through an AG 11 A8 (Bio-Rad) column. AG 11 A8 is an ion retardation resin that contains paired anion and cation exchange sites and can be used to remove SDS and other inorganic salts from proteins. In addition, optimum ampholyte combinations will be determined. The use of a zwitterionic detergent such as 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) instead of the non-ionic detergent NP-40 in the first dimension gel and sample buffers will also be investigated.

Table 1

Composition of Gel and Sample buffers Used for First Dimension Isoelectric Focusing

First dimension gel monomer solution

9.2 M urea
4 % acrylamide (total monomer)
2 % NP-40
1.6 % pH 5-7 ampholyte
0.4 % pH 3-10 ampholyte
0.01 % ammonium persulfate
0.1 % TEMED

First dimension sample buffer

9.5 M urea
2 % NP-40
5 % 2-mercaptoethanol
1.6 % pH 5-7 ampholyte
0.4 % pH 3-10 ampholyte

First dimension sample overlay buffer

9 M urea
0.8 % pH 5-7 ampholyte
0.2 % pH 3-10 ampholyte
0.0025 % bromophenol blue

Cathode buffer: 20 mM NaOH

Anode buffer: 10 mM H3PO4